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Rapid Whole-Genomic Sequencing and a Targeted Neonatal Gene Panel in Infants With a Suspected Genetic Disorder

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IMPORTANCE Genomic testing in infancy guides medical decisions and can improve health outcomes. However, it is unclear whether genomic sequencing or a targeted neonatal gene-sequencing test provides comparable molecular diagnostic yields and times to return of results.

OBJECTIVE To compare outcomes of genomic sequencing with those of a targeted neonatal gene-sequencing test.

DESIGN, SETTING, AND PARTICIPANTS The Genomic Medicine for Ill Neonates and Infants (GEMINI) study was a prospective, comparative, multicenter study of 400 hospitalized infants younger than 1 year of age (proband) and their parents, when available, suspected of having a genetic disorder. The study was conducted at 6 US hospitals from June 2019 to November 2021.

EXPOSURE Enrolled participants underwent simultaneous testing with genomic sequencing and a targeted neonatal gene-sequencing test. Each laboratory performed an independent interpretation of variants guided by knowledge of the patient's phenotype and returned results to the clinical care team. Change in clinical management, therapies offered, and redirection of care was provided to families based on genetic findings from either platform.

MAIN OUTCOMES AND MEASURES Primary end points were molecular diagnostic yield (participants with ≥1 pathogenic variant or variant of unknown significance), time to return of results, and clinical utility (changes in patient care).

RESULTS A molecular diagnostic variant was identified in 51% of participants (n = 204; 297 variants identified with 134 being novel). Molecular diagnostic yield of genomic sequencing was 49% (95% CI, 44%-54%) vs 27% (95% CI, 23%-32%) with the targeted gene-sequencing test. Genomic sequencing did not report 19 variants found by the targeted neonatal gene-sequencing test; the targeted gene-sequencing test did not report 164 variants identified by genomic sequencing as diagnostic. Variants unidentified by the targeted genomic-sequencing test included structural variants longer than 1 kilobase (25.1%) and genes excluded from the test (24.6%) (McNemar odds ratio, 8.6 [95% CI, 5.4-14.7]). Variant interpretation by laboratories differed by 43%. Median time to return of results was 6.1 days for genomic sequencing and 4.2 days for the targeted genomic-sequencing test; for urgent cases (n = 107) the time was 3.3 days for genomic sequencing and 4.0 days for the targeted gene-sequencing test. Changes in clinical care affected 19% of participants, and 76% of clinicians viewed genomic testing as useful or very useful in clinical decision-making, irrespective of a diagnosis.

CONCLUSIONS AND RELEVANCE The molecular diagnostic yield for genomic sequencing was higher than a targeted neonatal gene-sequencing test, but the time to return of routine results was slower. Interlaboratory variant interpretation contributes to differences in molecular diagnostic yield and may have important consequences for clinical management.

Multimedia

Supplemental content

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To improve clinical diagnosis in acutely ill neonates and infants, genomic sequencing must provide timely and accurate molecular diagnoses without the ethical burden of identifying unintended secondary findings.[1-12] Commercially available targeted-sequencing tests can interrogate a finite number of genes associated with specific genetic disorders. These panels are less expensive than genomic sequencing, return results faster, and rarely identify secondary findings.[13-16] A comparative analysis between genomic sequencing and a targeted neonatal gene-sequencing test has not been previously performed.[17]

The Genomic Medicine in Ill Infants and Newborns (GEMINI) study examined the time to diagnosis, molecular diagnostic yield, and clinical utility of genomic sequencing compared with a targeted neonatal gene-sequencing test in hospitalized infants younger than 1 year of age suspected of having a genetic disorder. In 2021, an unplanned interim analysis described results of the first 113 participants enrolled in GEMINI, motivated by the detection of 51 novel variants.[18] Here, specified analyses on all 400 participants are reported.

Key Points

Question How does molecular diagnostic yield and the time to return of results differ between genomic sequencing and a commercially available targeted neonatal gene-sequencing test in 400 hospitalized infants suspected of having a genetic disorder?

Findings Median time to result was 6.1 days for genomic sequencing and 4.2 days for the targeted gene-sequencing test. Molecular diagnostic yield of genomic sequencing was 49% (95% CI, 44%-54%) vs 27% (95% CI 23%-32%) with the targeted gene-sequencing test. Changes in clinical interventions affected 19% of participants.

Meaning In hospitalized infants, a genomic-sequencing approach achieved a higher molecular diagnostic yield but had a longer time to return of results than a commercially available targeted neonatal gene-sequencing test.

Methods

Study Design and Oversight

GEMINI was a prospective, comparative multicenter study with oversight provided by the principal investigators, the central (Johns Hopkins University) and local institutional review boards, and an independent data and safety monitoring committee.[18] Participating sites were Tufts Medical Center (Boston, Massachusetts), Rady Children's Hospital (San Diego, California), University of Pittsburgh Medical Center (Pittsburgh, Pennsylvania), Mount Sinai Kravis Children's Hospital (New York, New York), North Carolina Children's Hospital (Chapel Hill, North Carolina), and Cincinnati Children's Hospital Medical Center (Cincinnati, Ohio). Informed consent was obtained for each participant from their parent(s).

Participants

Participants were hospitalized infants younger than 1 year of age (corrected postmenstrual age) with a suspected genetic disorder and their parents, if available. Exclusion criteria were birth at less than 23 weeks' gestation, congenital infection, known genetic disorder explaining phenotypic findings, and infants not expected to receive all of their medical care in the United States. Testing was considered urgent if participants required mechanical ventilation, suffered from severe neurologic injury, were hemodynamically unstable, or if urgent testing was requested by the site principal investigator. Race and ethnicity were self-reported using closed categories. Use of *other* as a race and ethnicity category allowed for open responses from participants.

Though trio testing was preferred (proband, mother, father), enrollment depended on the proband. Parents could opt-in for their infant and themselves for secondary findings. Secondary findings were never sought but were a consequence of a phenotypically driven genomic interpretation.[19] Secondary findings for parents were only reported if listed on the American College of Medical Genetics gene list of reportable secondary findings.[19] Conversely, secondary findings for the proband followed guidelines established in newborn screening programs in which variants were reported if disease presentation occurred in childhood with an available treatment option. Nonpaternity was never revealed; incest involving a minor would be reported.

Sample Acquisition

Probands provided 1 mL of whole blood (shipped to Rady Children's Institute for Genomic Medicine, San Diego, California) for genomic sequencing and 5 dried blood spots on filter paper (Perkin Elmer Health Sciences [shipped to Athena Diagnostics]) for the commercially available-targeted neonatal gene-sequencing test (NewbornDx). Parents provided 3 mL of whole blood for analysis on both tests. Both testing sites are accredited by the College of American Pathologists, certified by Clinical Laboratory Improvement Amendments of 1988, and licensed by New York State.

Phenotypic Interpretation

Phenotypic interpretation of the genome used human phenotype ontology terms provided to laboratories. Human phenotype ontology terms were determined by 3 clinicians including 1 geneticist or genetic counselor. Pertinent demographic and clinical data for each participant were recorded. Most (79%) families met with a geneticist prior to enrollment.

Genomic-Sequencing Analysis and Interpretation

Genomic sequences were aligned to human genome assembly GRCh37 (hg19) and both nucleotide and structural variants identified with the DRAGEN platform (version 3.7, Illumina) as previously described.[20] The Fabric Enterprise Platform (Fabric Genomics) automatically annotated and ranked nucleotide and structural variants, which were manually interpreted by molecular geneticists according to published guidelines.[21,22] Preliminary written reports were issued for all provisional diagnoses. Results that could lead to an available treatment were immediately released to the site. All other variants determined to be potentially causative by either test were confirmed by Sanger sequencing, polymerase chain reaction, or chromosomal microarray before the report was finalized.

Targeted Neonatal Gene-Sequencing Test: Analysis and Interpretation

The targeted gene sequencing test has 1722 genes associated with disorders that typically present in early life. Genes on the targeted gene-sequencing test and the methodology used for DNA extraction and library preparation have been published.[18,23] Sequencing reads were mapped to the reference genome GRC37 (hg19) and sorted for variant calling using Edico DRAGEN version 2.6.5 (Illumina). Opal clinical software identified relevant variants with a standard framework used to assess candidate variants for pathogenicity. A variant scientist, molecular geneticist, and genetic counselor reported all identified variants. Reporting to sites was similar to genomic sequencing.

Variant Result Classification

Variants were classified as pathogenic, likely pathogenic, or as a variant of unknown significance (VUS). A VUS was only reported if it was located in or near a gene that was highly suspicious of causing the participant's phenotype. A suspicious VUS was defined as a heterozygous VUS that was reported as diagnostically important by laboratory directors. A suspicious VUS occurred in a compound heterozygous state with a pathogenic or likely pathogenic heterozygous variant in or near a gene associated with a disorder that fit the proband phenotype.

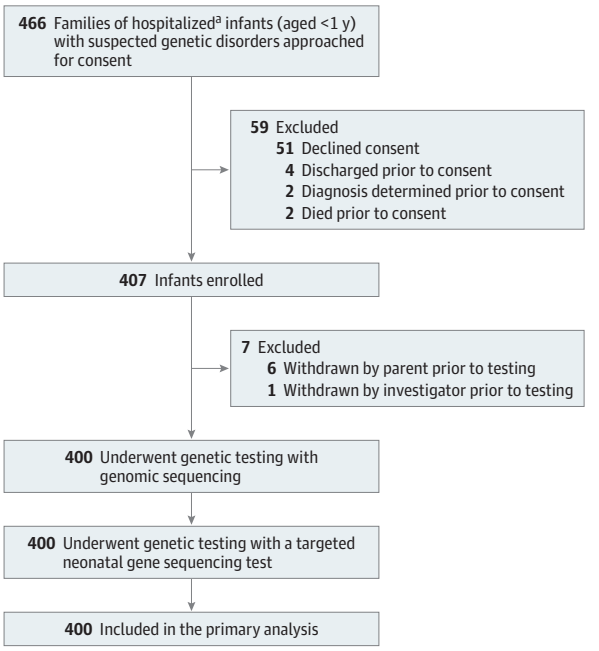
Noncoding variants were considered pathogenic or likely pathogenic if they were predicted to impact splicing. A noncoding VUS was considered pathogenic if it was predicted to impact splicing of exons critical for protein function associated with disorders that matched the phenotype. Large structural variants were considered pathogenic or likely pathogenic if they had previously been causally associated with a specific syndrome.

Although a VUS was considered to be a diagnostic finding, it was never referred to as an American College of Medical Genetics molecular diagnostic finding. A variant was considered novel if it had not been previously reported. All variants were uploaded to the ClinVar public archive (National Library of Medicine) and linked to the GEMINI study. Discordant variant reporting was defined as a discrepancy between pathogenic, likely pathogenic, VUS, variant not reported, or a difference in relationship to phenotype. With any discrepancy in interpretation, the study participant was placed into the highest level of variant classification. Clinical interpretation of variants and decisions to alter care were always performed by the infant's clinician, without regard to the specific test. Clinicians reported any changes in clinical management based on genetic results to the study team.

Outcomes

Primary end points included the molecular diagnostic yield of both tests, time to return of results (TTR), and clinical utility. Molecular diagnostic yield was defined as the number and associated percentage of participants with at least 1 pathogenic or likely pathogenic variant or a suspicious VUS. TTR was defined as the hours between receipt of the proband's sample and initial release of findings. Alterations in treatment strategies were made by the clinician based on results provided by either test. Clinical utility was defined by changes in medical, surgical, and/or nutritional management or changes in goals of care and measured by the physician of record (intensivist or geneticist) on a 5-point Likert scale (1 [not useful at all], 2 [not very useful], 3 [neutral], 4 [useful], 5 [very useful]). Physicians of record also rated the overall utility of genomic sequencing based on collective results from both tests. A secondary end point was report of secondary findings in probands and parents. Indeterminant results, unclassified or prognostic variants, and secondary findings were not included in the molecular diagnostic yield but were assessed as part of clinical utility.

Figure 1. Flow of Enrollment and Analysis in the GEMINI Study



[a] Eligible infants could have been recruited at any point of hospitalization during the first year of life (at birth or on subsequent admission) from Tufts Medical Center, Rady Children's Hospital, University of Pittsburgh Medical Center, Mount Sinai Kravis Children's Hospital, North Carolina Children's Hospital, and Cincinnati Children's Hospital Medical Center.

Statistical Analyses

A sample size of 400 infants produced adequate precision on the proportion of infants with a confirmed genetic diagnosis, with a width on the 2-sided Wilson score 95% CI to be less than or equal to 10% when the proportion was 50%. The proportion of participants with a confirmed genetic disorder was estimated for each platform, with a Wilson score 95% CI. Results from genomic sequencing and the targeted genomic-sequencing panel were cross-tabulated. McNemar odds ratios (ORs) were calculated for a confirmed genetic disorder identified with genomic sequencing compared with the targeted genomic-sequencing panel and its associated 95% CI. A McNemar OR greater than 1 indicated an increased odds of a confirmed genetic disorder with genomic sequencing compared with the targeted genomic-sequencing test. Bayesian methods were used to estimate the positive and negative diagnostic likelihood ratios, with the associated 95% credible intervals for genomic sequencing and the targeted genomic-sequencing test.[24,25]

Table 1. Baseline Characteristics of Infants With and Without a Confirmed Molecular Diagnosis (N = 400)[a]

	Infants with a confirmed molecular diagnosis[b] (n = 204)[c]	Infants without a confirmed molecular diagnosis (n = 196)[c]	Full analysis cohort (N = 400)[c]
Infant characteristics			
Gestational age, mean (SD), wk	36.7 (3.4)	36.5 (3.8)	36.6 (3.6)
Gestational age, wk			
<28	6 (2.9)	10 (5.1)	16 (4.0)
28-<34	24 (11.8)	27 (13.8)	51 (12.8)
34-<37	45 (22.1)	36 (18.4)	81 (20.3)
≥37	129 (63.2)	123 (62.8)	252 (63.0)
Age at enrollment, median (IQR), d	14.5 (7.0-53.0)	26.0 (9.0-83.0)	18.0 (8.0-66.5)
Age at enrollment, d			
≤30	127 (62.3)	105 (53.6)	232 (58.0)
31-60	31 (15.2)	26 (13.3)	57 (14.2)
61-90	17 (8.3)	22 (11.2)	39 (9.8)
91-120	9 (4.4)	12 (6.1)	21 (5.3)
≥121	20 (9.8)	31 (15.8)	51 (12.8)
Postmenstrual age at enrollment, mean (SD), d	299.2 (66.2) [n = 203]	316.7 (82.7)	307.8 (75.2) [n = 399]
Postmenstrual age at enrollment, wk			
<28	0	1 (0.5)	1 (0.3)
28-<33	10 (4.9)	2 (1.0)	12 (3.0)
33-<37	28 (13.8)	21 (10.7)	49 (12.3)
37-<44	116 (57.1)	108 (55.1)	224 (56.1)
44-<48	12 (5.9)	21 (10.7)	33 (8.3)
≥48	37 (18.2)	43 (21.9)	80 (20.1)
Race[d,e]	n = 174	n = 173	n = 347
American Indian	2 (1.1)	2 (1.2)	4 (1.2)
Asian	13 (7.5)	9 (5.2)	22 (6.3)
Black	18 (10.3)	25 (14.5)	43 (12.4)
Multiracial	27 (15.5)	36 (20.8)	63 (18.2)
White	109 (62.6)	99 (57.2)	208 (59.9)
Other	5 (2.9)	2 (1.2)	7 (2.0)
Hispanic ethnicity[d]	49 (25.8) [n = 190]	65 (35.7) [n = 182]	114 (30.6) [n = 372]
Female sex	95 (46.6)	74 (37.8)	169 (42.3)
Male sex	109 (53.4)	122 (62.2)	231 (57.8)
Pregnancy and birth history			
Assisted reproduction	9 (4.4)	9 (4.6) [n = 194]	18 (4.5) [n = 398]
Multiple gestation	10 (4.9)	16 (8.2) [n = 194]	26 (6.5) [n = 398]
Prior genetic consult	167 (81.9)	148 (75.5)	315 (78.8)
Consanguinity reported	10 (4.9)	5 (2.6)	15 (3.8)
Delivery method		n = 195	n = 399
Vaginal	85 (41.7)	83 (42.6)	168 (42.1)
Cesarean	119 (58.3)	112 (57.4)	231 (57.9)
Birthweight, mean (SD), g	2719.5 (894.1) [n = 203]	2689.0 (929.7) [n = 194]	2704.6 (910.7) [n = 397]
5-min Apgar score, median (IQR)	8.0 (7.0-9.0) [n = 188]	8.0 (7.0-9.0) [n = 176]	8.0 (7.0-9.0) [n = 364]
Enrolling site			
Enrolled at birth, same hospital	46 (22.5)	20 (10.2)	66 (16.5)
Enrolled after transfer from birth hospital	102 (50.0)	120 (61.2)	222 (55.5)
Enrolled after discharge home	56 (27.5)	56 (28.6)	112 (28.0)

(continued)

Table 1. Baseline Characteristics of Infants With and Without a Confirmed Molecular Diagnosis (N = 400)[a] (continued)

	Infants with a confirmed molecular diagnosis[b] (n = 204)[c]	Infants without a confirmed molecular diagnosis (n = 196)[c]	Full analysis cohort (N = 400)[c]
Maternal characteristics			
Age, mean (SD), y	30.4 (5.6)	30.0 (5.9) [n = 194]	30.2 (5.7) [n = 398]
Race[d,f]	n = 174	n = 173	n = 347
American Indian	2 (1.1)	3 (1.7)	5 (1.4)
Asian	15 (8.6)	13 (7.5)	28 (8.1)
Black	21 (12.1)	29 (16.8)	50 (14.4)
Multiracial	6 (3.4)	14 (8.1)	20 (5.8)
White	123 (70.7)	112 (64.7)	235 (67.7)
Other	7 (4.0)	2 (1.2)	9 (2.6)
Hispanic ethnicity[d,f]	47 (23.7) [n = 198]	57 (30.5) [n = 187]	104 (27.0) [n = 385]
Paternal characteristics			
Race[d,g]	n = 167	n = 172	n = 339
American Indian	3 (1.8)	3 (1.7)	6 (1.8)
Asian	13 (7.8)	11 (6.4)	24 (7.1)
Black	22 (13.2)	33 (19.2)	55 (16.2)
Multiracial	7 (4.2)	7 (4.1)	14 (4.1)
Pacific Islander	0	1 (0.6)	1 (0.3)
White	115 (68.9)	114 (66.3)	229 (67.6)
Other	7 (4.2)	3 (1.7)	10 (2.9)
Hispanic ethnicity[d,g]	39 (20.9) [n = 187]	54 (29.3) [n = 184]	93 (25.1) [n = 371]

[a] Numeric values are reported as No. (%) unless otherwise indicated.

[b] Confirmed molecular diagnosis indicates at least 1 genetic variant identified by either testing modality that was deemed either diagnostic or highly suspicious of causing the presenting phenotype.

[c] Indicates the categorical number of participants unless otherwise indicated.

[d] Race and ethnicity were self-reported using closed categories except for *Other*, which allowed for open responses from participants. Multiple selection was not allowed, but participants could select *Multiracial*. Families were asked to report race and ethnicity, but denominators may not total because some families reported ethnicity but not race, and some reported neither.

[e] If the father's race was not reported, the infant's race was reported as being the same as the mother's race; otherwise, the infant's race was recorded as the reported races of both parents.

[f] For maternal race, specific responses provided by participants for *Other* included Amish, Bangladeshi, Cape Verde, Dominican, Malian, Puerto Rican, and Puerto Rican/Taino.

[g] For paternal race, specific responses provided by participants for *Other* included Amish, Asian Black, Bangladeshi, Black African American, White Caucasian, Cape Verde, Dominican, Egyptian, Puerto Rican.

TTR for each test was summarized by the median (IQR). Median differences were estimated by the Hodges-Lehmann estimator with 95% CIs. Clinical utility was assessed by examining changes in clinical care by frequencies and percentages. All analyses were performed with Stata version 17.0.

Results

Recruitment occurred from June 2019 to November 2021. The study included 400 probands, and 388 mothers and 318 fathers consented to the study and provided blood for analysis (**Figure 1**). Clinical and demographic data of participants are provided in **Table 1**. Most participants were male (57.8%), born at 37 weeks' gestation or longer (63.0%), and delivered via cesarean (57.9%). Assisted reproductive technologies (4.5%) and multiple pregnancies (6.5%) were above national means.[26,27]

Among 400 infants, 204 participants (51% [95% CI, 46%-56%]) had at least 1 genetic variant identified by either test that was deemed as causal (either pathogenic or likely pathogenic) or highly suspicious (VUS) of causing the presenting phenotype (**Figure 2**A). Nine participants had suspicious VUS identified in noncoding regions, and 8 participants had large structural variants identified. Genetic variants were identified in 195 participants (49% [95% CI, 44%-54%]) by genomic sequencing and 109 participants (27% [95% CI, 23%-32%]) by the targeted genomic-sequencing panel (Figure 2B). Among infants with a genetic variant (VUS) identified, 49 of 195 (25%) were with genomic sequencing and 29 of 109 (27%) were with a targeted genomic-sequencing panel. Ninety-five infants had a diagnosis identified by genomic sequencing that was not detected with the targeted genomic-sequencing test (49% of the 195 positive cases by genomic sequencing). Nine infants had a diagnosis identified by the targeted genomic-sequencing test and not detected by genomic sequencing (8% of the 109 positive cases by the targeted genomic-sequencing test). This resulted in a McNemar OR for genomic sequencing vs the targeted genomic-sequencing test of 10.6 (95% CI, 5.3-23.8), indicating a 10-fold increase in the odds of a molecular diagnosis with genomic sequencing compared with the targeted genomic-sequencing test. The diagnostic likelihood ratios for genomic sequencing and the targeted genomic-sequencing test are reported in the eTable in Supplement 1. Among 107 urgent cases (eFigure 1 in Supplement 1) and variants identified

Figure 2. Comparison of Results Between Whole Genomic Sequencing and a Targeted Neonatal Gene-Sequencing Test at the Infant and Variant Levels

A 400 Included in the infant-level analysis

		Genomic sequencing			
		None/secondary[a]	Variants of unknown significance	Likely pathogenic or pathogenic variants	All infants
Targeted neonatal gene sequencing test	None/secondary[a]	196	35	60	291
	Variants of unknown significance	5	13	11	29
	Likely pathogenic or pathogenic variants	4	1	75	80
	All infants	205	49	146	400

B 518 Included in the variant-level analysis

		Genomic sequencing			
		None/secondary[a]	Variants of unknown significance	Likely pathogenic or pathogenic variants	All variants
Targeted neonatal gene sequencing test	None/secondary[a]	221	78	86	385
	Variants of unknown significance	14	22	11	47
	Likely pathogenic or pathogenic variants	5	1	80	86
	All variants	240	101	177	518

[a] None indicates that no variants were detected. Secondary refers to variants not associated with phenotype. Light blue cells indicate that a variant was detected by both laboratories and interpreted similarly by both laboratories; beige indicates that a variant was detected by both laboratories but classified differently; brown indicates that variants were only detected by one laboratory or that a variant was classified as related to phenotype by one laboratory and as a secondary finding by the other laboratory.

Table 2. Breakdown of Discordant Variant Findings

	Targeted neonatal gene-sequencing test did not report (n = 164)	Genomic sequencing did not report (n = 19)	Both platforms reported variant (n = 12)[a]	Overall (n = 195)
Inability to detect				
Structural variant >1 kilobase	49			49
Gene not included on targeted panel	48			48
Coverage of gene	4			4
Mitochondrial genome	4			4
Variant interpretation				
Interpretation	57	14	12	83
Filtering	1	2		3
Technical difference[b]	1	2		3
Mosaicism		1		1

[a] Variant was reported by both platforms but interpreted differently (pathogenic or likely pathogenic vs variant of unknown significance).

[b] Indicates a difference for one variant that drove interpretation difference on another variant.

(eFigure 2 in Supplement 1), the molecular diagnostic yields were 59 (55% [95% CI, 46%-64%]) by genomic sequencing and 35 (33% [95% CI, 25%-42%]) by the targeted genomic-sequencing test (McNemar OR for genomic sequencing vs the targeted genomic-sequencing test, 9.0 [95% CI, 2.8-46.4]).

Overall, 297 molecular diagnostic pathogenic or likely pathogenic variants or suspicious VUS were identified by genomic sequencing, the targeted genomic sequencing test, or both, with 134 (45% [95% CI, 40%-51%]) considered novel. Genomic sequencing did not report 19 variants found by the targeted genomic-sequencing test. The targeted genomic-sequencing test did not report 159 variants found by genomic sequencing and did report 5 variants as secondary findings (genomic sequencing deemed those same variants as diagnostic [n = 164]), giving a McNemar OR of 8.6 (95% CI, 5.4-14.7). Twelve variants were identified by both laboratories but classified by 1 laboratory as a VUS and the other laboratory as a pathogenic/likely pathogenic variant (**Table 2**). Among the 195 discordant variants, the targeted genomic-sequencing test was unable to detect structural variants longer than 1 kilobase (25%) or variants in genes not on the panel (25%). However, when both laboratories were able to detect the same variant, interpretation and classification of the variant differed 43% of the time (Table 2). The remaining discordances were due to overall gene coverage (2%), computational filtering (1.5%), mosaicisms (0.5%), mitochondrial gene variants (2%), and technical interpretation (1 variant drove the detection of another variant 1%). Six participants who had a preexisting variant not thought to explain the phenotype

Table 3. Time to Return of Results by Type of Test

	Targeted neonatal gene-sequencing test			Genomic sequencing		
	Indication of molecular diagnosis (n = 109)	Without indication of molecular diagnosis (n = 291)	Full analysis cohort (N = 400)	Indication of genetic disorder (n = 195)	Without indication of genetic disorder (n = 205)	Full analysis cohort (N = 400)
Time to return of results, median (IQR), h	100.6 (69.9-121.1)	100.3 (56.4-122.2)	100.3 (56.9-121.7)	101.7 (53.3-176.0)	170.1 (122.8-269.9)	146.6 (79.3-237.8)
Urgent cases (n = 107)[a]						
Time to return of results, median (IQR), h	76.7 (55.1-104.0) [n = 35]	99.6 (56.1-103.5) [n = 72]	98.5 (55.8-104.0) [n = 107]	53.5 (35.6-148.7) [n = 59]	99.9 (74.8-132.9) [n = 48]	79.9 (49.8-143.2) [n = 107]

[a] Urgent cases required mechanical ventilation, had signs of severe neurologic injury, or were hemodynamically unstable. Urgent testing could also be requested by the site principal investigator.

Table 4. Clinical Utility of Genomic Sequencing

	No. (%)		
	Confirmed molecular diagnosis (n = 204)[a]	Without confirmed molecular diagnosis (n = 196)	Full analysis cohort (N = 400)
Changes in management or in goals of care[b]			
Any	64 (31.4)	12 (6.1)	76 (19.0)
Medication	36 (17.6)	8 (4.1)	44 (11.0)
Surgery	20 (9.8)	3 (1.5)	23 (5.8)
Withdrawal of life-sustaining support	12 (5.9)	0	12 (3.0)
Diet	6 (2.9)	1 (0.5)	7 (1.8)
Change in goal of care from comfort to cure	3 (1.5)	0	3 (0.8)
Other changes in care			
Testing or screening	138 (67.6)	80 (40.8)	218 (54.5)
Specialty services[c]	71 (34.8)	24 (12.2)	95 (23.8)
Imaging	32 (15.7)	8 (4.1)	40 (10.0)
Perceived clinical utility[d]		n = 194	n = 398
Very useful	126 (61.8)	24 (12.4)	150 (37.8)
Useful	51 (25.0)	101 (52.1)	152 (38.2)
Neutral	20 (9.8)	40 (20.6)	60 (15.1)
Not very useful	7 (3.4)	24 (12.4)	31 (7.8)
Not useful at all	0	5 (2.6)	5 (1.3)

[a] Indicates at least 1 genetic variant identified by either testing modality that was deemed either diagnostic or highly suspicious of causing the presenting phenotype.

[b] Changes were determined via follow-up with the physician of record (intensivist or geneticist).

[c] Indicates neurology, endocrinology, and cardiology.

[d] Physicians of record rated the overall utility of the genomic-sequencing process (ie, based on collective results from both platforms) using a 5-point Likert scale (1, [not useful at all] to 5 [very useful]).

had their variants confirmed; only 2 of these infants had additional variants reported. By providing at least 1 parent sample, 87% of all variant inheritance (nondiagnostic, diagnostic) could be determined by genomic sequencing (eTable in Supplement 2). A description of variants, inheritance patterns, and associated disorders is provided in the eTable in Supplement 2.

Across all 400 participants, median TTR was 146.6 (IQR, 79.3-237.8) hours for genomic sequencing and 100.3 (56.9-121.7) hours for the targeted genomic-sequencing test (**Table 3**). The median difference between the 2 tests was 61.2 (95% CI, 37.9-85.6) hours. Among 107 urgent cases, median TTR was 79.9 (IQR, 49.8-143.2) hours for genomic sequencing and 98.5 (IQR, 55.8-104.0) hours for the targeted genomic-sequencing test (Table 3).

Changes in medical, surgical, and/or nutritional management occurred in 76 participants (19% [95% CI, 15%-23%]; **Table 4**). Fifteen infants transitioned from palliative care to either a known treatment for the disorder or withdrawal of life support. Six infants received changes in clinical management based solely on a VUS. Most physicians (302 of 398; 76% [95% CI, 71%-79%]) perceived genomic sequencing as useful or very useful, whether a diagnosis was provided or not (Table 4).

Among 374 infants eligible to receive secondary findings, 24 variants (6% [95% CI, 4%-9%]) were identified. Twelve mothers and 15 fathers who had the same autosomal dominant pathogenic or likely pathogenic variant as the proband (previously unknown). Three mothers and 1 father had variants associated with an increased risk of cancer and received genetic counseling.

Discussion

The comparative GEMINI study was conducted to assess the molecular diagnostic yield and TTR between genomic sequencing and a targeted neonatal-genomic-sequencing test. Although standard turnaround time was shorter for the targeted genomic-sequencing test, there was a trade-off in molecular diagnostic yield (27% vs 49%). Genomic sequencing returned results sooner in urgent cases with a similar molecular diagnostic

yield (49% vs 55%). Although these results highlight the feasibility of genomic sequencing and targeted genomic-sequencing tests to return results in clinically relevant time frames, caution should be taken with extrapolating these data to real-word settings. Access to the technology and well-developed study protocols likely affected the speed in which a molecular diagnosis was made.

GEMINI identified 134 novel variants in genes directly related to phenotype; 73 potentially causal variants were classified as a VUS per American College of Medical Genetics guidelines.[21] In this analysis, these variants were included in the diagnostic yield. While this is not standard, both laboratories indicated confidence that a suspicious VUS was highly likely to be related to the phenotype and should be reported to the clinical care team to inform care. Care was altered in 6 participants based solely on a suspicious VUS. Although American College of Medical Genetics regulations were established to limit premature linkage of a variant with causative phenotypes, our perspective is that these guidelines should not negate or minimize the potential determination of pathogenicity of a highly suspicious VUS.

With the integration of genomic technologies into pediatric care, clinicians will identify novel pathogenic variants that better inform the prevalence of genetic disorders. For example, 6 enrolled participants were diagnosed with Kabuki syndrome, which is estimated to occur in 1 of 32 000 to 86 000 live births.[28,29] In addition, 56% of infants with intrauterine growth restriction (n = 37) and/or microcephaly (n = 20) had a molecular diagnosis supporting a genetic disorder. Compared with national statistics,[25,26] GEMINI participants were more likely to be male, preterm, a multiple gestation, delivered by cesarean, and the product of assisted reproductive technologies. Only 33% of the consulting geneticists' pretest differential diagnoses correctly listed the genetic disorder identified by molecular testing. This highlighted the lack of distinct features of many neonatal genetic conditions and the limited knowledge regarding genetic disorders that present early in life. In this study, 22% of identified molecular diagnoses involved structural variants that may not have a well-defined phenotype. Genomic sequencing may provide the best opportunity for early identification of these as well as single gene disorders using a single test. As the use of these tests becomes more common, selection criteria for genomic testing should remain broad and inclusive.

Discordant variant identification and classification between laboratories occurred despite sharing identical DNA samples and clinical information. Most discrepancies were due to the inherent technical limitations of the targeted genomic-sequencing test. However, more than 40% of incongruent variant reporting was due to algorithmic identification and human interpretation. Our study is not the first to report on discrepant variant interpretation. The National Human Genome Research Institute's Clinical Sequencing Evidence-Gathering consortium published research regarding discrepant results in 2016 and 2020.[30,31] Assumptions regarding the accuracy of variant calls remain dependent on interpretation of the role of the variant in the clinical presentation of the proband, highlighting the need for continuous feedback and improvement of diagnostic algorithms. Caution should be used by clinicians when interpreting molecular diagnostic results from any single genomic-sequencing platform.

Although direct clinical interventions were implemented for only 19% of infants, most physicians viewed the testing as useful or very useful in their clinical decision-making, even when a nondiagnostic result was returned. GEMINI confirms the need to have such testing widely available and covered by Medicaid and commercial insurance. Additionally, earlier diagnoses may lead to new therapeutics. These technologies hold great promise for improving access to pharmacologic, biologic, and gene therapies in high-risk populations.

Limitations

This study has several limitations including the fact that the study did not formally assess whether the differences in molecular diagnostic yield translated to improvements in clinical outcomes. No formal superiority testing was conducted to assess whether the molecular diagnostic yield of one test was statistically superior to the other. The molecular diagnostic yield may also be increased due to the inclusion of suspicious VUS, which may, in future studies, be shown to be benign.

Conclusions

The molecular diagnostic yield for genomic sequencing was higher than that for a targeted neonatal gene-sequencing test, but overall TTR was slower. Interlaboratory variant interpretation contributes to differences in diagnostic yield and may have important consequences for clinical management and the development of future therapeutic interventions.

ARTICLE INFORMATION

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